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Body composition in anorexia nervosa: meta-analysis and meta-regression of cross-sectional and longitudinal studies

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Abbreviations: AN, anorexia nervosa; BIA, bioelectrical impedance analysis; DSM, Diagnostic and Statistical Manual of Mental Disorders; DXA, dual-energy X-ray absorptiometry; fT_3, free triiodothyronine; fT_4, free thyroxine; HDL, high-density lipoprotein; IBW, ideal body weight; ICD, International Classification of Diseases, MRI, magnetic resonance imaging; TSH, thyroid-stimulating hormone

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Abstract (236/250)

Objective: Clinically, anorexia nervosa (AN) presents with altered body composition. We quantified these alterations and evaluated their relationships with metabolites and hormones in AN patients longitudinally.

Method: In accordance with PRISMA guidelines, we conducted 88 meta-analyses on 60 samples published 1996-2018, comparing up to 2,294 pre-treatment, post-treatment, and weight-recovered AN patients with up to 1,848 controls. Primary outcomes were fat mass, fat-free mass, body fat percentage, and their regional distribution. Secondary outcomes were bone mineral density, metabolites, and hormones. Meta-regressions examined relationships among those measures and moderators.

Results: Pre-treatment AN patients evidenced 50% lower fat mass (MD: -9.05 kg, CI 95%: -10.02, -8.07, $Q = 9.33 \times 10^{-73}$) and 4.96 kg (CI 95%: -5.84, -4.07, $Q = 9.30 \times 10^{-27}$) lower fat-free mass, with fat mass preferentially stored in the trunk region during early weight restoration (4.2%, CI 95%: -2.1, -6.2, $Q = 2.30 \times 10^{-4}$). While the majority of traits returned to levels seen in healthy controls after weight restoration, fat-free mass (MD: -1.82 kg, CI 95%: -2.57, -1.08, $Q = 5.41 \times 10^{-6}$) and bone mineral density (MD: -0.10 kg, CI 95%: -0.18, -0.03, $Q = 0.01$) remained significantly altered.

Discussion: Body composition is markedly altered in AN, warranting research into these phenotypes as clinical risk or relapse predictors. Notably, the long term altered levels of fat-free mass and bone mineral density suggest that these parameters should be investigated as potential AN trait markers.

Introduction

Anorexia nervosa (AN) has one of the highest mortality rates of all psychiatric disorders (Chesney, Goodwin, & Fazel, 2014). Clinical observations show altered body composition (El Ghoch, Calugi, Lamburghini, & Dalle Grave, 2014; Solmi et al., 2016) accompanied by elevated cholesterol (Hussain et al., in press) and greater insulin sensitivity (Ilyas et al., 2018). However, conclusions are limited by small sample sizes and consequent mixed findings.

Molecular genetic studies have revealed that individuals with AN carry genetic variants that increase their liability to AN and concurrently predispose to lower body fat percentage, lower fasting insulin, and higher high-density lipoprotein (HDL) cholesterol concentrations, suggesting that metabolic factors may play an etiological role (Duncan et al., 2017; Watson et al., 2018). Additionally, longitudinal investigations of a British birth cohort showed that girls who develop AN later in life are already underweight at the age of 4 years when compared to healthy children (Yilmaz, Gottfredson, Zerwas, Bulik, & Micali, in press), adding evidence for a developmental component.

A systematic review showed that adolescents and adults differently lose fat tissue when affected by AN, with adolescents losing more central fat tissue and adults more peripheral fat tissue. During weight recovery, individuals with AN show emergent central adiposity which typically attenuates over time (El Ghoch, Calugi, et al., 2014). These clinical and genetic findings encourage the meta-analytic reassessment of the role of body composition traits, such as fat mass and fat-free mass, their regional distribution, and their changes associated with weight restoration and long-term weight recovery in AN.

The goals of these meta-analyses were to (1) replicate findings from the systematic review on fat mass; (2) extend the observations by quantifying them; (3) include fat-free mass and (4) bone mineral content and density; (5) investigate their associations with each other;

and (6) if possible, relate findings to secondary outcomes, such as metabolic and hormonal parameters. This analytical approach is aimed at understanding the potential associations between these factors that are known to be physiologically interrelated. A thorough and rigorous examination of body composition and related laboratory parameters in individuals with AN could elucidate some of the physiological changes associated with this serious disorder, which could lead to more effective medical management, monitoring, and treatment approaches.

Methods

Search strategy

Our meta-analysis was conducted according to PRISMA guidelines (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009) and pre-registered (PROSPERO 2018 CRD42018105338) with no changes to the protocol. We conducted a literature search from 15.06.2018 until 01.07.2018 using the electronic database PubMed with a time limitation starting with articles published after 01.01.1994—marking the introduction of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV; American Psychiatric Association, 2013)—until 31.06.2018. We used key search terms including "anorexia nervosa" AND ("body composition" OR "body fat" OR "fat mass" OR "body fat percentage" OR DXA OR BIA OR "fat free mass" OR "lean mass"). The search was repeated by co-authors to avoid selection bias. Furthermore, we screened the references of published articles and reviews. Our search results including the selection process are presented in Supporting Information Figure S1 according to PRISMA guidelines.

Selection criteria

Our inclusion criteria were as follows:

a. Studies investigating humans only
b. Any age group
c. No sample overlap
d. Observational cross-sectional or longitudinal studies or randomised-controlled trials
e. Clinical diagnoses of AN according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV, 5, or their revisions (American Psychiatric Association, 2013), or International Classification of Diseases version 10 (ICD-10) (World Health Organization, 1992)
f. Investigation of body composition by dual-energy X-ray absorptiometry (DXA) (Bredella et al., 2010, 2013), bioelectrical impedance analysis (BIA) (Bonaccorsi et al., 2012; Mattar et al., 2011), dual photon absorptiometry (DPA), or magnetic resonance imaging (MRI) (Mayer et al., 2005).
g. Published after 01.01.1994 (the year that DSM-IV was introduced)
h. The study includes a control group or comparison group
i. Publications in any language which could be translated by the research team: English, German, Swedish, Danish, Spanish

In case of multiple publications deriving from the same study population, we selected the articles reporting either the largest or the most recent data set. In case of conflict between these two criteria, large sample size was prioritized.

Data extraction

We extracted the following information from every identified study using a standardised data extraction sheet:

a. Author, publication year
b. Country
c. Sample sizes including gender and age
d. Setting: inpatient or outpatient
e. Original longitudinal or cross-sectional design
f. Follow-up period if longitudinal
g. Diagnostic criteria: DSM-IV, DSM-IV-TR, DSM-5, or ICD-10
h. Participant screening and exclusion criteria
i. Number of cases: AN pre-treatment, post-treatment (ANpost), recovered from AN (ANrec)
j. Subtype of AN: restricting (R), binge-eating/purging (BP)
k. Number of controls (CO)
l. Primary outcome variables of body composition: fat mass (FM), fat-free mass (FFM), body fat percentage (BF%), and their regional distribution
m. Secondary outcome variables, which were reported by at least four studies additional to primary outcomes: bone mineral density (BMD), glucose, insulin, ghrelin, adiponectin, leptin, insulin-like growth factor (IGF-1), estradiol, testosterone, cortisol, thyroid-stimulating hormone (TSH), free triiodothyronine (fT_3), free thyroxine (fT_4)
n. Covariates used in original analysis
o. Fasting and fasting duration
p. Blood sample: serum, plasma, or unspecified
q. Medication and contraceptives

r. Psychological and additional treatments
s. Outcome was a secondary or primary outcome in the original study
t. Duration of illness
u. Age at diagnosis/onset
v. Age at menarche
w. Percentage of AN cases with amenorrhea and duration of amenorrhea

Quality of study assessment (Newcastle-Ottawa Scale)

We used the Newcastle-Ottawa Scale (NOS) to assess the quality of studies (Wells et al., 2009). For the observational studies, low quality was defined as Newcastle–Ottawa Scale score ≤8.0 and high quality as score >8.0 (maximum score 9).

Meta-analysis

Inverse variance-weighted meta-analyses were conducted using the statistical package 'meta' and 'metafor' in the open source software R v3.5.1 (r-project.org). We used additional formulas to calculate missing values (Hozo, Djulbegovic, & Hozo, 2005; Luo, Wan, Liu, & Tong, 2018; Wan, Wang, Liu, & Tong, 2014). Mean differences (MDs) between clinical and control groups were the effect sizes. A random-effects model, which assumes that the heterogeneity in the differences between clinical and control groups is due to both within-study and between-study variation, was used as we anticipated differences in procedures and study populations between studies. We used a restricted maximum-likelihood estimator (REML) to calculate the heterogeneity. For the analysis of subtypes, post-treatment, and weight-recovered AN patients, the control groups from the acutely-ill/pre-treatment analysis were reused because (1) control groups were not measured repeatedly; and (2) none of the studies had separate control groups for each subtype analysis. Although some studies

included covariates in their statistical analysis (Bratland-Sanda et al., 2010; Bredella et al., 2008; Dellava, Policastro, & Hoffman, 2009; DiVasta et al., 2007; Fernández-Soto, González-Jiménez, Chamorro-Fernández, & Leyva-Martínez, 2013; Haas et al., 2005; Karlsson, Weigall, Duan, & Seeman, 2000; Kosmiski, Schmiege, Mascolo, Gaudiani, & Mehler, 2014; Maïmoun et al., 2018; Nakahara et al., 2007; Schneider et al., 1998), we only used raw values without including study-specific covariates to increase comparability across individual studies. Weight recovery was defined in accordance with DSM-IV and DSM-5 criteria with BMI >18.5 kg/m^2 or >90% ideal body weight (IBW). To correct our primary analysis for multiple testing, False Discovery Rate-adjusted *Q values* were calculated (Benjamini & Hochberg, 1995).

Publication bias or small study effects

Meta-analyses should diminish both bias and uncertainty of the overall estimate; however, this is not always the case as published studies may represent a biased selection of evidence (Nieminen, Rucker, Miettunen, Carpenter, & Schumacher, 2007). To detect publication bias, we inspected funnel plots visually (Egger, Smith, Schneider, & Minder, 1997) and quantified their asymmetry using a weighted linear regression by Thompson and Sharp—which allows for between-study heterogeneity (Thompson & Sharp, 1999). The significance of this test ($p < 0.05$) indicates asymmetric funnel plots and suggests publication bias or small study effects. In this case, we fitted a Copas selection model to adjust our meta-analysis for these effects.

Copas selection model

The Copas selection model to correct for potential selection bias comprises: (1) a model for the meta-analyzed effect; and (2) a model estimating the probability that study *k* is selected for publication. The correlation parameter ρ between these two components indicates the extent of publication bias with larger correlations suggesting more extreme effects have been selected for publication (Copas, 1999; Copas & Shi, 2000, 2001). We used the R package 'metasens' which fits the Copas selection model repeatedly over a grid of tuning parameters γ_0 and γ_1 and creates a standardised output: a) a funnel plot, b) a contour plot, c) a treatment effect plot, and d) a p value plot.

Meta-regression and stratified subgroup analysis

We detected extensive heterogeneity across the studies (Table 1). The heterogeneity observed in a meta-analysis can be partially systematic and related to clinical study-level variables (i.e., moderators), such as age or AN subtype. In secondary analyses, potential moderators were therefore explored in stratified subgroup analyses, such as AN subtype, or via meta-regression analyses, including mean age, the time period of follow-up for longitudinal studies, age at diagnosis, age at menarche, age at amenorrhea, duration of illness, percentage of amenorrhea in AN patients, percentage of medicated AN patients, percentage of individuals taking contraceptives, body composition measurement method, blood sample type, body composition parameters and their differences between cases and controls. Mixed effects models allow for within-study and between-study variation and were therefore the most appropriate model indicated by the large heterogeneity estimates detected in our primary meta-analyses. We used a restricted maximum-likelihood (REML) estimator to calculate the heterogeneity included in the R package 'meta'.

Results

Results of the search and selection of studies

A total of 510 papers published between 1996 and 2018 were identified by our search terms, and 450 of them were excluded. No paper published 1994-1996 fulfilled the inclusion criteria and the most common reasons for exclusion were: (1) no control group (n = 137); (2) sample overlap (n = 65); and (3) no main outcome reported (i.e., body composition; n = 45). Detailed exclusion process is presented in Supporting Information Figure S1. Sixty published articles were included in our analysis (Agüera et al., 2015; Bachmann et al., 2014; Benninghoven, Raykowski, Solzbacher, Kunzendorf, & Jantschek, 2007; Bratland-Sanda et al., 2010; Bredella et al., 2012, 2008; Chudecka & Lubkowska, 2016; de Alvaro et al., 2007; Dellava et al., 2009; Delporte, Brichard, Hermans, Beguin, & Lambert, 2003; de Mateo Silleras et al., 2013; Diamanti et al., 2007; DiVasta et al., 2007, 2011; Dostálová, Sedlácková, Papezová, Nedvídková, & Haluzík, 2009; El Ghoch et al., 2012, 2015; El Ghoch, Calugi, Milanese, Bazzani, & Dalle Grave, 2017; El Ghoch, Milanese, et al., 2014; El Ghoch, Pourhassan, et al., 2017; Estour et al., 2017; Faje et al., 2014; Fazeli et al., 2010; Fernández-Soto et al., 2013; Galusca et al., 2015; Germain et al., 2010, 2007, 2016; Gniuli, Liverani, Capristo, Greco, & Mingrone, 2001; Grinspoon et al., 1996, 2001; Guo, Jiang, Liao, Liu, & He, 2013; Haas et al., 2018, 2005; Iacopino et al., 2003; Karczewska-Kupczewska et al., 2010; Karlsson et al., 2000; Kaválková et al., 2012; Kerruish et al., 2002; Kirchengast & Huber, 2004; Konstantynowicz et al., 2011; Kosmiski et al., 2014; Maïmoun et al., 2018; Mayer et al., 2009, 2005; Mika, Herpertz-Dahlmann, Heer, & Holtkamp, 2004; Misra et al., 2013; Moreno, Djeddi, & Jaffrin, 2008; Mörkl et al., 2017; Nakahara et al., 2007; Nakai, Hamagaki, Takagi, Taniguchi, & Kurimoto, 1999; Prioletta et al., 2011; Rigaud, Boulier, Tallonneau, Brindisi, & Rozen, 2010; Scalfi, Marra, Caldara, Silvestri, & Contaldo, 1999; Scalfi et al., 2002; Schneider et al., 1998; Singhal et al., 2018; Tagami et al., 2004; Tanaka et

al., 2003; Weinbrenner et al., 2004), and we became aware of no additional unpublished samples after contacting study authors for additional or missing data (Supporting Information Table S1). The majority of studies focused on female cases and controls that were sampled consecutively in only 21 of 60 studies (Supporting Information Table S2) and aged between 14.2 and 31.0 years (Supporting Information Figure S2). As such, two studies investigating male AN cases were excluded from the qualitative synthesis but are discussed briefly (El Ghoch, Calugi, et al., 2017; Misra et al., 2013). Three studies originated from Australasia, 37 from Europe, 15 from North America, and five from Asia. Only 13 studies used the same method of ascertainment for cases and controls (Supporting Information Table S2). Twenty-eight studies investigated inpatients, eight outpatients, two a mixture of both, and 22 studies did not specify the recruitment or patient setting. All studies comprised collection of blood samples after a fasting period, whereas only six studies specified the fasting period (Bredella et al., 2012; DiVasta et al., 2011; Dostálová et al., 2009; Estour et al., 2017; Kaválková et al., 2012; Prioletta et al., 2011). One study did not specify whether analyses were performed using plasma or serum blood (Weinbrenner et al., 2004). Seventeen studies sampled regular menstruating participants during the follicular phase of their cycle (de Alvaro et al., 2007; Dostálová et al., 2009; Estour et al., 2017; Galusca et al., 2015; Germain et al., 2010, 2007, 2016; Kaválková et al., 2012; Kirchengast & Huber, 2004; Mayer et al., 2005; Nakai et al., 1999; Prioletta et al., 2011; Weinbrenner et al., 2004), whereas 13 studies did not provide details about cycle phase (Bachmann et al., 2014; Bredella et al., 2012; Delporte et al., 2003; DiVasta et al., 2011; Fazeli et al., 2010; Fernández-Soto et al., 2013; Germain et al., 2010; Gniuli et al., 2001; Grinspoon et al., 1996; Guo et al., 2013; Haas et al., 2005; Karczewska-Kupczewska et al., 2010; Maïmoun et al., 2018; Mörkl et al., 2017; Nakahara et al., 2007; Rigaud et al., 2010; Tagami et al., 2004; Tanaka et al., 2003). However, studies were retained to achieve the largest possible sample size, and—depending on data availability—meta-

regressions were fitted to investigate study characteristics as possible moderators. Originally, 39 studies were cross-sectional and 21 longitudinal (Supporting Information Table S1). However, three of the longitudinal studies were analysed cross-sectional in our meta-analysis due to missing data. No control group was repeatedly measured in any of the longitudinal studies.

Characteristics of the included studies

We performed four meta-analyses: 1) comparing 2,697 pre-treatment/acutely-ill AN patients with 2,251 healthy controls; 2) comparing 722 post-treatment AN patients with 809 controls; 3) estimating the change in AN patients (n = 722) from pre- to post-treatment; and 4) comparing 398 weight-recovered individuals with AN with 660 healthy controls including samples with long-term follow-up. The pre-treatment AN group comprised 229 individuals suffering from the binge-eating/purging and 681 from the restricting subtype. The shortest follow-up period was 5.14 weeks, and the longest was 2 years (Supporting Information Table S1). Nineteen studies used BIA to assess body composition, 38 used DXA, and only three utilized MRI—considered to be the gold standard. Thirty of the 60 studies investigated body composition as a primary outcome, whereas it was a secondary outcome in the remaining studies. The percentage of AN patients with amenorrhea ranged from 43 to 100%, with ten studies not providing information on menstrual status (Agüera et al., 2015; Bachmann et al., 2014; Bredella et al., 2012; de Mateo Silleras et al., 2013; El Ghoch et al., 2012; Gniuli et al., 2001; Iacopino et al., 2003; Kirchengast & Huber, 2004; Schneider et al., 1998; Tagami et al., 2004; Tanaka et al., 2003). Thirty-five of 60 studies did not provide information on the medication status of AN patients, and 30 did not indicate whether oral contraceptives were used. In AN cases, the duration of illness was on average 52.59 months (SD = 29.08), the duration of amenorrhea 22.99 months (SD = 18.27), and the age at diagnosis 17.45 years (SD

315 = 3.01). Cases and controls were well matched for age (Supporting Information Figure S2)
316 and, notably, we did not observe a difference in age at menarche (Supporting Information
317 Figure S3) or height (Supporting Information Figure S6) between AN cases and controls.
318
319 **Data and analyses results of meta-analyses and meta-regressions**
320 Our results from the 88 meta-analyses show that a wide range of alterations in several
321 key body composition and biochemical measures exist in AN cases compared with healthy
322 controls (Figure 1/Supporting Information Figure S4). For 95% confidence intervals and Q
323 values, heterogeneity estimates, and adjusted estimates due to estimated publication bias, see
324 Table 1. Detailed forest plots showing each of the 88 meta-analyses are presented as
325 Supporting Information Figures S5-92. No differences between restricting and binge-
326 eating/purging subtype of AN were detected in our meta-analysis prior to treatment except
327 for total body water (Supporting Information Table S3). Between-study heterogeneity (I^2)
328 was observed in 62 meta-analyses and ranged from 52 to 99%, confirming our choice of a
329 random-effects model. To investigate moderators implicated in heterogeneity, we performed
330 398 meta-regressions (Supporting Information Table S4-7). Eight meta-analyses showed
331 funnel plot asymmetry, indicating small study effects. Therefore, we fitted Copas models to
332 adjust for those effects and estimate the probable number of unpublished studies (Table 1 and
333 Supporting Information Figure S93-100).
334
335

Primary outcomes: body composition

Anthropometrics

On average, pre-treatment AN cases had a 15.61 kg (CI 95%: -16.98, -14.24, $Q = 7.26 \times 10^{-109}$) lower body weight and were 0.01 m (CI 95%: -0.01, 0.00, $Q = 0.03$) shorter than healthy controls given their age ($\beta_{metareg} = -0.002$, $p = 0.04$;Supporting Information Table S4). After treatment, AN patients still weighed 4.92 kg (CI 95%: -8.03, -1.81, $Q = 1.92 \times 10^{-3}$) less than healthy controls.

Correspondingly, the pre-treatment BMI difference between AN cases and controls was -5.88 kg/m^2 (CI 95%: -6.31, -5.46, $Q = 9.06 \times 10^{-158}$), which reduced to -2.10 kg/m^2 (CI 95%: -2.53, -1.67, $p_{adjCopas} < 1.00 \times 10^{-4}$) after treatment as most patients gained on average 9.93 kg (CI 95%: 8.17, 11.68, $Q = 2.11 \times 10^{-27}$) during treatment. Post-treatment BMI was primarily accounted for by gains in fat mass ($\beta_{metareg} = 0.81$, $p = 7.03 \times 10^{-7}$) but not through fat-free mass (Supporting Information Table S5). After weight recovery, no statistically significant mean difference in BMI between AN cases and controls was detected.

Fat mass

The pre-treatment body composition of individuals with AN was significantly altered. Compared with healthy controls, AN cases had 9.05 kg (CI 95%: -10.02, -8.07, $Q = 9.33 \times 10^{-73}$) lower fat mass, corresponding to a 13.9% (CI 95%: -15.2, -12.6, $Q = 9.59 \times 10^{-99}$) lower complete body mass. This suggests that body fat was on average 50% lower than in healthy controls. Body fat percentage ($\beta_{metareg} = -134.53$, $p = 0.01$) and absolute fat mass ($\beta_{metareg} = -49.26$, $p = 1.65 \times 10^{-4}$) were associated with whole body bone mineral density of AN patients. Absolute fat mass was also associated with mean age at diagnosis ($\beta_{metareg} = -1.21$, $p = 2.42 \times 10^{-4}$; Supporting Information Table S4).

After treatment, AN patients had a 2.37 kg (CI 95%: -3.75, -0.98, $Q = 0.002$) lower fat mass which corresponded to 2.5% (CI 95%: -4.3, -0.7, $p_{adjCopas} = 0.006$) less total body mass compared with healthy controls. AN patients gained 6.39 kg (CI 95%: 5.13, 7.65, $Q = 3.07 \times 10^{-22}$) fat mass following treatment which corresponded to 10.4% (CI 95%: 7.96, 12.87, $Q = 6.25 \times 10^{-16}$) of total body mass. Post-treatment fat mass ($\beta_{metareg} = -0.23$, $p = 0.01$; Supporting Information Table S5) and gain in fat mass during treatment ($\beta_{metareg} = -0.20$, $p = 0.02$; Supporting Information Table S6) were negatively associated with the presence of amenorrhea. Following weight recovery, these values fully returned to levels seen in healthy controls.

Specifically, compared with healthy controls, AN patients had 3.51 kg (CI 95%: -4.58, -2.43, $Q = 8.07 \times 10^{-10}$) less trunk fat mass prior to treatment. In relative terms, however, AN patients had lower extremity body fat with 5.4% (CI 95%: -8.38, -2.43, $Q = 8.23 \times 10^{-4}$) less total body mass. The presence of amenorrhea was significantly associated with lower extremity fat mass ($\beta_{metareg} = 0.31$, $p = 0.04$; Supporting Information Table S4).

After treatment, AN patients showed a higher trunk body fat percentage than controls at 12.0% (CI 95%: 9.5, 14.4, $p_{adjCopas} < 1.00 \times 10^{-4}$) of total body mass. However, this finding was strongly influenced by publication bias with an estimated 52 unpublished studies. These results on body composition were not influenced by height as cases and controls showed no meaningful difference (i.e., 1 cm pre-treatment) or by age as meta-regressions were non-significant (Supporting Information Tables S4-7).

Fat-free mass

Overall, the fat-free mass in AN patients was 4.96 kg (CI 95%: -5.84, -4.07, $Q = 9.30 \times 10^{-27}$) lower pre-treatment than in controls, corresponding to 12.3% (CI 95%: 8.1, 16.5, $Q = 3.07 \times 10^{-8}$) higher proportion of total body mass. During treatment, AN patients gained 2.98 kg (CI 95%: 1.74, 4.22, $Q = 6.89 \times 10^{-6}$) fat-free mass, resulting in 1.82 kg (CI 95%: -2.57, -1.08, $Q = 5.41 \times 10^{-6}$) lower fat-free mass compared to controls. Yet, weight-recovered individuals with AN still showed 1.27 kg (CI 95%: -1.79, -0.75, $Q = 5.49 \times 10^{-6}$) lower fat-free mass than controls.

More specifically, pre-treatment fat-free mass of the extremities was 1.5% (CI 95%: -2.0, -1.0, $Q = 8.84 \times 10^{-9}$) less of total body mass. After treatment, no marked regional differences in fat-free mass were observed in AN patients. However, weight-recovered individuals with AN had 0.9% (CI 95%: -1.3, -0.5, $Q = 6.30 \times 10^{-5}$) lower trunk fat-free mass of total body mass than controls.

Before treatment, we observed a 393.95 kcal/day (CI 95%: -531.04, -256.86, $Q = 6.27 \times 10^{-8}$) lower resting energy expenditure and 4.65 L (CI 95%: -6.21, -3.10, $Q = 1.78 \times 10^{-8}$) less total body water in AN patients which persisted with 3.71 L (CI 95%: -7.07, -0.36, $Q = 0.05$) after treatment. Both were measured by BIA. However, resting energy expenditure was not corrected for fat-free mass or body mass in the original studies, limiting its interpretability. The association between total body water and fat-free mass could not be investigated as too few studies reported both outcomes concurrently.

Before treatment, only the amount of total body water was significantly different between individuals ($p_{subgroup} = 0.004$) suffering from the restricting (-5.21 L, CI 95%: -9.06, -1.36, $p_R = 8.04 \times 10^{-3}$, $k = 3$) or the binge-eating/purging subtype (-11.1 L, CI 95%: -12.04, -

10.16, p_{BP} = 5.06 x 10^{-119}, k = 1; Supporting Information Table S3). However, this finding was limited by only one study investigating the binge-eating/purging subtype.

Secondary outcome: bone mineral measures

Bone mineral content and density

Compared with healthy controls, whole body bone mineral content in individuals with AN was 0.16 kg (CI 95%: -0.19, -0.12, Q = 2.73 x 10^{-20}) lower pre-treatment and 0.09 kg (CI 95%: -0.13, -0.05, Q = 1.63 x 10^{-5}) lower post-treatment. Weight-restored individuals with AN showed 0.10 kg (CI 95%: -0.18, -0.03, Q = 0.01) lower whole body bone mineral content compared to controls as they gained on average 0.05 kg (CI 95%: 0.02, 0.09, Q = 0.01) during treatment. The interpretability of these estimates is limited due to the insufficient follow-up time after weight recovery, exceeding six months in only two studies (Dellava et al., 2009; Karlsson et al., 2000). The pre-treatment whole body bone mineral content was associated with fat-free mass ($\beta_{metareg}$ = 0.02, p = 0.02) and fat mass ($\beta_{metareg}$ = 0.05, p = 0.02), as well as the difference in fat mass between AN patients and controls ($\beta_{metareg}$ = 0.04, p = 0.002; Supporting Information Table S4). Accordingly, whole body pre-treatment bone mineral density was 0.03 g/cm^2 (CI 95%: -0.07, -0.00, $p_{adjCopas}$ = 0.02) lower in AN, but our analysis showed a density comparable with healthy controls post-treatment. However, only two studies with 74 AN cases could be included in this analysis.

Before treatment, AN patients exhibited lower bone mineral density in several regions, including hip, lumbar spine, and femoral neck, with a few being likely to persist after weight recovery. These findings were associated with duration of illness, the age of AN cases, and differences in fat mass between cases and controls (Supporting Information: Secondary outcomes: detailed bone mineral measures and Table S4). Cases and controls in

our meta-analyses were age- and height-matched (Supporting Information Figure S2 & S6), therefore, these variables are unlikely to be associated with these results.

Secondary outcomes: metabolites and hormones

Exploratory results showed that fasting insulin and glucose concentrations were lower in AN patients compared with controls but not associated with fat or fat-free mass while lower leptin was associated with fat mass pre-treatment. After treatment, these measures returned to concentrations seen in healthy controls. Before treatment, thyroid hormones, cortisol, and IGF-1 were lower in AN patients and all three measures were associated with fat mass whereas higher cortisol in AN patients was associated with fat-free mass. For detailed results see Supporting Information: Secondary outcomes: metabolites and hormones.

Methodological moderators

We observed strong between-study heterogeneity (Table 1). To investigate how differences in study design, samples, and measurement methods may influence the primary and secondary outcomes, we performed an additional set of meta-regressions (Supporting Information Tables S4-7). The method of body composition measurement was associated with pre-treatment body fat percentage (β_{DXA} = 3.34, p = 0.008), fat-free mass ($\beta_{Isotope\ Dilution}$ = -6.18, p = 0.009), and fat-free mass percentage (β_{DXA} = -8.28, p = 0.01) and post-treatment body fat percentage (β_{DXA} = 6.39, p = 0.005) . Furthermore, femoral neck bone mineral density ($\beta_{Outpatient}$ = -0.12, p = 7.65 x 10^{-4}) significantly differed between inpatients and outpatients.

Discussion

Our primary meta-analyses showed marked alterations in body composition traits in patients with AN before and after treatment. Before treatment, all three major body compartments—fat, fat-free, and bone mass—showed significant reductions that were only partially restored after treatment. Our meta-analysis estimated ~50% lower body fat in AN patients which was mirrored by leptin concentrations (Perry & Shulman, 2018), both of which recovered with treatment. Significant differences were observed in body fat distribution post-treatment as body fat was primarily stored in the trunk. This distribution pattern may be due to increased insulin sensitivity observed in AN patients (Ilyas et al., 2018) potentially similar to observations in healthy individuals after short-term overfeeding (McLaughlin et al., 2016). We did not detect meaningful or statistically significant differences in body fat distribution in weight-restored patients, indicating potential redistribution occurring over longer term follow-up.

A new finding from our meta-analysis is that lower fat mass in AN was correlated with significantly low bone mineral content and density across the whole body. We speculate that the hormonal cross-talk between fat and bone tissue may be influencing this association (El Ghoch et al., 2016; Hawkes & Mostoufi-Moab, 2018), potentially mediated through greater bone marrow adipose tissue observed in AN (Fazeli & Klibanski, 2018; Suchacki & Cawthorn, 2018). Whole body bone mineral content remained low in weight-recovered individuals with AN. However, as only two studies followed patients for longer than six months (Dellava et al., 2009; Karlsson et al., 2000), the length of follow-up was insufficient to draw firm conclusions because bone mineral mass is slow to normalize. Future studies should be designed to capture long term changes. In men with AN, it has been reported that short-term weight restoration may normalize body composition patterns but could also lead to central adiposity (El Ghoch, Calugi, et al., 2017). However, sample sizes of reports of males

are very small. Additionally, in our analysis alterations in bone mineral mass did not affect the height of individuals with AN.

Another new finding in our meta-analysis is that we observed a 5 kg lower fat-free mass in AN patients which remained lower even after treatment and in weight-recovered AN patients, indicating that current treatment regimes may insufficiently target fat-free mass. Future studies should also assess muscle mass to identify the components of fat-free mass that are most associated with this reduction.

Our secondary outcomes—associations between detailed body composition and laboratory parameters in AN—were difficult to assess as only a few published studies reported both outcomes consistently. Most biochemical alterations were within the range of normal reference values. However, serious alterations can occur in certain individuals with AN that warrant vigilance by clinicians.

Overall, the meta-analyzed study sample was highly selected and biased as it comprised mostly European females aged between 14 and 31 years, emphasising the urgent need for studies including diverse ancestries, such as Asia, South and Central America, and Africa. Females and males differ in body composition and metabolic characteristics (Karastergiou & Fried, 2017; Link & Reue, 2017), underscoring the need for more studies on males with AN. Our study selection was limited by lack of control groups and underreported extensive sample overlap. Moreover, control groups were only measured at baseline in all longitudinal studies, failing to account for age- and growth-related variation, potentially inflating estimates. Furthermore, no clear-cut and consistent definition of recovery from AN was used across studies, contributing to heterogeneity (Murray, Loeb, & Le Grange, 2018). This underscores the necessity of developing standard definitions of remission and recovery in the eating disorders field (Bardone-Cone, Hunt, & Watson, 2018).

Methodologically, we observed effects of either BIA or DXA on the measurement of body composition in AN, questioning the comparability of the two methods. Larger, longitudinal validation studies comparing both methods with whole body MRI in eating disorders should be conducted. Additional factors influencing body composition and biochemical measures, such as menstrual cycle, diurnal changes, fasting, and pre-analytical procedures are summarised in Table 2 and should be carefully assessed in future studies (Hernandes, Barbas, & Dudzik, 2017).

Most importantly, blood is comprised of approximately 3,500 highly correlated and interacting proteins (hupo.org) (Schwenk et al., 2017) and 4,600 metabolites (serummetabolome.ca) (Psychogios et al., 2011), therefore, the measurement of single proteins, hormones or metabolites is ill-advised. Metabolomics, proteomics, and lipidomics can capture large amounts of information at adequate statistical power when used in large samples (Hernandes et al., 2017). Additionally, large epidemiological databases that have measured biomarkers in childhood, such as the Avon Longitudinal Study of Parents and Children (ALSPAC; Golding, Pembrey, Jones, & ALSPAC Study Team, 2001) and Generation R (Kooijman et al., 2016), should be harnessed to determine whether those who go on to develop AN show evidence for premorbid differences in body composition and biochemical parameters as has been observed for BMI by Yilmaz et al. (Yilmaz et al., in press).

Conclusions

Detailed measurement of body composition with simple methods, such as BIA or DXA, that offers additional information on bone tissue, may help refine our understanding of the nature of AN and its diagnosis. Our meta-analyses showed that all body compartments were markedly altered in AN. Individuals with AN presented with 50% lower fat mass and prolonged recovery periods for fat-free mass and bone mineral content. The core implication of body composition differences are alterations in metabolism, growth, and development. While results must be interpreted with caution given small samples, we found evidence indicating alterations in fasting insulin, thyroid, sex, and stress hormones in AN which appeared to partially normalize with weight gain and recovery. Large birth cohorts that collected information on eating disorders along with metabolomic information offer a rich and exciting opportunity for prospective investigations that add to our understanding of body composition and metabolic mechanisms in risk and maintenance of eating disorders.

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Conflict of interest

Dr. Bulik reports: Shire (Scientific Advisory Board member) and Pearson (author, royalty recipient) (unrelated to the content of this manuscript). Dr. Breen has received grant funding from and served as a consultant to Eli Lilly and has received honoraria from Illumina and has served on advisory boards for Otsuka (all unrelated to the content of this manuscript). The remaining authors declare that they have no conflict of interest.

Authors' contributions

CH, ZY, CMB and GB designed research; CH, ZY, KS, LB, AH, JGG conducted research; CH, ZY, KS, LB, AH, JGG provided essential materials; CH analyzed data or performed statistical analysis; CH, ZY, LB, KS, GB, CMB wrote paper; CH had primary responsibility for final content. All authors read and approved the final manuscript.

Data availability

All data and all scripts used for data analysis are available on

github.com/topherhuebel/metabcan

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867 **Table 2** Minimum requirement of variables that should be assessed, reported, and included in
868 statistical analyses of case-control studies examining anorexia nervosa or other eating
869 disorders to facilitate reproducibility, meta-analysis, and -regression [adapted from, 111]

Sampling	**Sample characteristics**
Cases and controls ● Underlying population: community, hospital ● Consecutive sample or selection ● If consecutive, attrition & reasons ● Diagnosis & ascertainment ● Diagnostic schema ● Independent validation Controls ● Repeated measurement ● Exclusion of current and history of diagnosis (i.e., screening) ● Matching (e.g., age, sex) ● Exclusion criteria	Cases and controls ● Age ● Biological sex or gender ● Height ● Weight ● Body mass index ● Ancestry ● Socioeconomic status Cases ● Age of onset ● Duration of illness
Body composition	**Menstrual status**
● Fat mass ● Fat-free mass ● Bone mineral content & density ● Ideally: muscle mass	Cases ● Dysmenorrhea or amenorrhea ● Duration of amenorrhea ● Age of menarche

• Measurement method: MRI, DXA, or BIA • Physical activity (ideally accelerometer data)	• If menstruating, stage or day of cycle Controls • Stage or day of the menstrual cycle (e.g., follicular phase)
Blood sampling	**Substances**
• Blood sample type Whole blood, serum, plasma • Fasting state • Fasting period • The time point of blood sampling • Pre-analytics • Storage • Storage duration	Dose and duration of • Contraceptives • Supplements & vitamins • Medication ○ Prescription ○ Over the counter • Laxatives • Illicit drugs • Alcohol consumption • Smoking behaviour
BIA, bioelectrical impedance analysis, DXA, dual-energy X-ray absorptiometry, MRI, magnetic resonance imaging	

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